

Appl. No. 09/843,462
Response dated January 29, 2004
Reply to Office Action of July 29, 2003

Amendments to the Specification:

Please delete the paragraph beginning at page 7, line 21 to page 8, line 9, which starts with "After the CDK-phosphorylated Rb protein" and replace with the following amended paragraph:

After the CDK-phosphorylated Rb protein in the test sample is complexed with the capture antibody, the complex is isolated from the remaining sample. To isolate this complex, it is preferred that the capture antibody is first attached to a solid phase support, for example, a multi-well plate or a bead, by standard methods known in the art (see, e.g., Current Protocols in Immunology, John Wiley and Sons, New York, NY, 1999). Examples of solid phase supports that can be used for antibody attachment include, in order of preference, ~~Nunc Maxisorb~~ Nunc-Maxisorb™ plates, ~~Nunc-Polyserb~~ Nunc-Polysorb™ plates, and protein A coated plates (VWR Scientific, Bridgeport, NJ). To attach the capture antibody to the solid phase support, the capture antibody is diluted in a buffer such as 0.05 M carbonate-bicarbonate pH 9.6 (Cat. # C-3041, Sigma Chemical Co., St. Louis, MO) to approximately 10 µg/ml. This antibody dilution is then applied to the plate and incubated for a time ranging from approximately 4 hours to overnight at 4°C. The antibody-bound support is washed in a 25 mM ~~Trizma~~ Trizma® base (Tris) or a 50 mM N-(2-hydroxyethyl)-N'-(2-ethanesulfonic acid) (Hepes) based buffer (pH 7.4-7.8) containing salt (e.g., 100-150 mM NaCl) and a small amount of detergent (e.g., 0.02-0.1% ~~Tween-20~~ Tween-20™) (all of the wash buffer components are available from Sigma Chemical Co., St. Louis, MO). An exemplary wash buffer is an imidazol wash buffer (Cat. # 50-63-01, KPL, Inc., Gaithersburg, MD). (In the event that an alkaline phosphatase detection system is used in a subsequent step of this assay, phosphate buffered saline is not recommended as an incubation or wash buffer.)

Please delete the paragraph beginning at page 8, line 10, which starts with "Following the wash step," and replace with the following amended paragraph:

Following the wash step, the capture antibody-bound support is incubated with a blocking buffer for approximately 0.5-2 hours at room temperature. Exemplary blocking buffers include, in order of preference, Superblock #37515 (Pierce Chemical, Rockford, IL), SuperBlock #37535 (Pierce Chemical, Rockford, IL), 0.2% Casein (Sigma Chemical Co., St. Louis, MO) in Tris buffered saline (TBS) (137 mM NaCl, 3 mM KCl, 25 mM Tris Base pH7.6; all components available from Sigma Chemical Co., St. Louis, MO) and 0.1% ~~Tween-20~~ Tween-20™, and 0.05% skim milk in TBS and 0.1% ~~Tween-20~~ Tween-20™ (all of the incubation buffer components are available from Sigma Chemical Co., St. Louis, MO). Subsequent to the aspiration of the blocking buffer from the capture antibody-bound support, the next step is contact with the test sample.

Please delete the paragraph beginning at page 11, line 18, which starts with "Whether the cell lysate is derived from cultured cells" and replace with the following amended paragraph:

Whether the cell lysate is derived from cultured cells or a tissue sample, the cell lysate is added to the capture antibody-bound plates and is incubated for approximately 1-3 hours at room temperature. It is preferred that all incubations are performed on a low speed shaker at 30-200 revolutions/minute. The cell lysate is aspirated and an anti-Rb primary antibody,

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diluted to approximately 1-5 µg/ml in an incubation buffer such as 1% casein in TBS pH 7.4-7.8, with or without 0.1% ~~Tween-20~~ Tween-20TM, is added to the capture antibody-bound plates.

Please delete the paragraph beginning at page 16, line 17, which starts with "A solution containing a capture antibody" and replace with the following amended paragraph:

A solution containing a capture antibody diluted to 10 µg/ml in a 0.05 M carbonate-bicarbonate buffer pH 9.6 (Cat. #C3041, Sigma Chemical Co., St. Louis, MO) was applied to coat the wells of 96-well plates (100 µl/well, ~~Nunc F96 MaxiSorp Nunc-MaxisorbTM~~, VWR Scientific, Bridgeport, NJ) and incubated overnight at 4°C. For CDK2 assays, anti-phosphoRb antibody, which specifically recognizes Rb phosphorylated at Ser807 and Ser811, was used (Cat. #9308L, New England ~~Biolabs~~ Biolabs®, Inc., Beverly, MA); for CDK4 assays, anti-phosphoRb antibody, which specifically recognizes Rb phosphorylated at Ser 780, was used (Cat. #9307S, New England ~~Biolabs~~ Biolabs®, Inc., Beverly, MA, or Code #555, Medical and Biological Laboratories, Nagoya, Japan).

Please delete the paragraph beginning at page 18, line 3, which starts with "The plates, with bound capture antibody-Rb complexes," and replace with the following amended paragraph:

The plates, with bound capture antibody-Rb complexes, were incubated with an anti-Rb monoclonal antibody (phosphorylation-independent) that recognizes an Rb epitope between amino acids 300-380 (Cat. # 14001A, ~~PharMingen~~ PharMingen®, San Diego, CA). This primary antibody was diluted to 2 µg/ml in 1% casein, TBS pH 7.6, 0.1% Tween-20 (all incubation buffer components are available from Sigma Chemical Co., St. Louis, MO). One hundred microliters of the solution was added to each well, and the plates were then incubated on the shaker at the low speed setting for two hours at room temperature. The plates were then washed six times with the imidazol wash buffer to isolate the capture antibody-Rb-primary antibody complex bound to the plate.

Please delete the paragraph beginning at page 18, line 12, which starts with "The final antibody incubated on the plate" and replace with the following amended paragraph:

The final antibody incubated on the plate was a donkey anti-mouse alkaline phosphatase-labelled secondary antibody (Cat. #715-055-150, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). The secondary antibody was diluted 1:2000 in the incubation buffer consisting of 1% casein, in TBS pH 7.6, 0.1% ~~Tween-20~~ Tween-20TM, and 100 µl of the solution was added to each well. Following the incubation of the plates on the shaker at the low speed setting for one hour at room temperature, the plates were again washed six times with the imidazol wash buffer to isolate the capture antibody-Rb-primary antibody-secondary antibody complex.